Chronic ingestion of dietary fat is a prerequisite for inhibition of feeding by enterostatin

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Lin, Ling, and David A. York. Chronic ingestion of dietary fat is a prerequisite for inhibition of feeding by enterostatin. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R619–R623, 1998.—Enterostatin (Ent), the activation pentapeptide from procolipase, inhibits the intake of dietary fat. The selectivity of the response to fat suggests that the rat must recognize a permissive signal related to dietary fat for the Ent biological response. To investigate the nature of this signal, we studied the effects of Ent in rats that were adapted to either a high-fat (HF) or high-carbohydrate/low-fat (HC) diet and then naively exposed to either HF or HC diets. Ent (1 nmol) was injected into the lateral ventricle of overnight-fasted rats, and food intake was measured. Rats adapted to HF diet and tested with HC diet responded to Ent, but rats adapted to HC diet and tested with HF did not respond to Ent. The groups were maintained on their new test diets for up to 21 days and tested again for their response to Ent at 3, 7, 14, and 21 days. Ent response did not appear in HC-adapted rats switched to HF diet before 21 days. Conversely, the HF-adapted rats, which responded to Ent when tested with HC diet for the first time, did not respond at any subsequent testing time. The data suggest that chronic ingestion of dietary fat is required for Ent action and that chronic consumption of fat initiates a postigestion metabolic, endocrine, or neurochemical change that is required for the biological response to Ent.

chronic fat ingestion; food intake

ENTEROSTATIN (Ent) is the NH₂-terminal pentapeptide released from pancreatic procolipase by trypsin during fat ingestion, in which colipase serves as a cofactor of intestinal fat digestion (12, 14). Studies in rats, cats, and humans have shown that Ent concentrations increased in intestinal content, lymph, and circulation after a fat meal (4, 24, 36). Adaptation to diets high in fat is associated with increased synthesis of pancreatic (28) and gastric procolipase (J. Chen and D. A. York, unpublished observations), the parent molecule of Ent. Furthermore, rats that display a high voluntary intake of fat have a low content of pancreatic procolipase consistent with feedback regulation of fat intake by Ent (30).

A number of studies have shown that exogenous Ent selectively inhibits the intake of dietary fat after peripheral or central injections (13, 19, 20, 29). The peripheral response to Ent is mediated via the afferent vagus because both vagotomy and capsaicin treatment abolis its feeding suppression (35, 37). Centrally, Ent interacts with both opioid and serotonergic pathways to inhibit fat intake (3, 22, 31). Localized injection studies have shown that Ent is effective in both the amygdala and the paraventricular nucleus but not in the ventromedial hypothalamus or the nucleus of the solitary tract (23). However, all reports on Ent inhibition of feeding, after either central or peripheral administration or after chronic or acute administration, have been made in the animals that were first adapted to diets rich in fat content before testing. A number of experimental feeding regimes have been used for testing the response to Ent. After adaptation to a three-choice macronutrient diet, Ent reduces intake of fat but not of carbohydrate or protein (25, 30); on a two-choice high-fat (HF) and high-carbohydrate/low-fat (HC) diet, Ent reduces intake of the HF but not the HC diet (18, 31). Finally, Ent only inhibits the intake in single-choice diet when it is high in fat content and does not reduce intake of an HC diet (19, 20, 30).

The mechanism through which Ent inhibits intake of dietary fat is not known. It is clear that the rat must recognize the presence of fat in the diet for this macronutrient-selective effect to work, i.e., there must be a secondary permissive signal related to dietary fat for the Ent biological response. To understand whether this cognitive ability is dependent on an immediate signal or a gustatory, gastrointestinal, or postabsorptive signal associated with ingestion of fat or requires chronic adaptive changes, we compared the responses to Ent in rats naively exposed to either HF or HC diets with those of rats that were adapted to HC or HF diets before testing.

METHODS

Animals and experimental diets. Male Sprague-Dawley rats were purchased from Harlan Industries (Indianapolis, IN) at 8 wk of age with an initial body weight of 180–200 g. They were individually housed in hanging stainless steel wire mesh cages under controlled temperature (22–23°C) and lighting (0700–1900) conditions with free access to an automatic watering system. Experimental diets were formulated to provide different proportions of calories derived from fat and carbohydrate as previously described (18). These diets provided either 56% energy as fat (HF diet) or 66% carbohydrate and 10% fat by energy (HC diet) and were isocaloric in protein content (24% by energy). They were fed for 14 days before surgery. Food cups were secured in the front of each cage with a stainless steel spring. Fresh diet was provided daily.

Intracerebroventricular cannulation. Rats weighing 270–300 g were anesthetized with pentobarbital sodium (40 mg/kg) and stereotaxically implanted with a chronic 22-gauge
stainless steel guide cannula into the right lateral cerebral ventricle. The coordinates were 1.4 mm lateral to midsagittal, 0.8 mm posterior to bregma, and 3.5 mm ventral to the dura, according to the atlas of Paxinos and Watson (33). Cannulas were secured in place with anchor screws and dental acrylic and occluded with 26-gauge obturators. The positions of each cannula were determined at the end of the experiment by injection of 5 µl of india ink through the guide cannula, after which the brain was dissected to examine for ink in the ventricle. All of the rats in these studies had cannulas correctly implanted.

Ent injection. Ent was synthesized by the Core Laboratory at Louisiana State University Medical School (New Orleans, LA). The purity of the Ent (>92%) was checked by reverse-phase HPLC and mass spectrometry. Ent was dissolved in sterile saline (0.9% wt/vol) vehicle and injected into the lateral ventricle at a dose of 1 nmol in 5 µl vehicle as previously reported (20). All central injections were performed with a Harvard infusion pump through a 26-gauge injector that extended 0.5 mm beyond the tip of the guide cannula.

Procedures. The rats were allowed to recover from surgery for 1 wk before testing. On the experimental day, rats were deprived of food overnight for 20 h, with water available, before Ent (1 nmol) or vehicle injection at 1200. After injection, rats were returned to their home cages and provided with either their habitual diet or the alternative diet. Food intake was recorded at 0.5-, 1-, 2-, and 4-h time points and corrected for spillage. Thus four separate groups of rats were used for these experiments as follows: 1) rats adapted to HF diet and tested with an HF diet, 2) rats adapted to HF diet and tested with the HC diet, 3) rats adapted to HC diet and tested with HC diet, and 4) rats adapted to HC diet and tested with HF diet. After the initial test, all rats were subsequently maintained and restested on their test meal diets. For example, rats adapted to the HF diet that were tested on HF diet continued on the HF diet (group 1), whereas those rats (group 2) that were initially tested on the HC diet were subsequently maintained and restested on the HC diet. The responses to Ent were studied in the same group of rats on days 1, 3, 7, 14, and 21 of adaptation to the test diets.

Data analysis. The results of food consumption were expressed as means ± SE by weight (g). Data were statistically evaluated by ANOVA (1-way or 2-way), with main effects for drug or time (h) and repeated measures (days) as appropriate. Individual differences between the vehicle and treatment groups were compared by Duncan post hoc tests. Each group included at least five rats. Statistical significance was considered P < 0.05.

Table 1. Food intake of HF or HC diet by rats previously adapted to same diets

<table>
<thead>
<tr>
<th>Time, h</th>
<th>HF Diet (g)</th>
<th>Ent (g)</th>
<th>HC Diet (g)</th>
<th>Ent (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.9 ± 0.3</td>
<td>2.9 ± 0.2*</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>6.6 ± 0.2</td>
<td>4.9 ± 0.2*</td>
<td>3.7 ± 0.5</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>7.9 ± 0.7</td>
<td>6.7 ± 0.4</td>
<td>6.4 ± 0.4</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>8.1 ± 0.7</td>
<td>6.9 ± 0.3</td>
<td>8.8 ± 1.0</td>
<td>7.4 ± 0.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean cumulative food intake (g) ± SE. Ent, enterostatin; HF, high fat; HC, high carbohydrate. ANOVA indicated significant effects of diet and treatment (diet F1,19 = 10.49, P < 0.005 and treatment F1,19 = 8.87, P < 0.01). There were no interactions between diet and treatment (F1,19 = 1.62, P = 0.22). *P < 0.05 vs. corresponding saline treatment.

Table 2. Food intake of HC diet by rats previously adapted to HF diet

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Day 1 (g)</th>
<th>Day 3 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Ent</td>
</tr>
<tr>
<td>0.5</td>
<td>3.9 ± 0.3</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>1</td>
<td>5.6 ± 0.2</td>
<td>4.5 ± 0.1*</td>
</tr>
<tr>
<td>2</td>
<td>6.9 ± 0.5</td>
<td>5.2 ± 0.2*</td>
</tr>
<tr>
<td>4</td>
<td>7.9 ± 0.5</td>
<td>5.7 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are expressed as mean cumulative food intake (g) ± SE. There were significant main effects of day (F1,24 = 15.57, P < 0.001) and treatment (F1,24 = 6.52, P < 0.02). Day × treatment interaction was not significant. (F1,24 = 1.9, P = 0.18). *P < 0.05 vs. corresponding saline treatment.
were maintained on these test diets, and diet. The effects of Ent on food intake of rats that were tested on their habitual diet and retested on subsequent days as shown; n = 7 for each group. ANOVA indicated a significant effect of day (F(4,54) = 5.22; P < 0.001) but not treatment (F(1,54) = 1.32; P = 0.26) or interaction day × treatment (F(4,54) = 0.71; P = 0.59). *P < 0.05 compared with saline group.

RESULTS

Test diets identical to habitual diet. The effects of Ent on food intake of rats that were tested on their habitual diet are shown in Fig. 1 and Table 1. Ent inhibited the intake of HF diet in rats that were adapted previously to the HF diet by 25% 1 h after injection of Ent (saline 6.6 ± 0.2 g vs. Ent 4.9 ± 0.2 g, P < 0.05). In contrast, Ent had no effect on intake of HC test diet in rats previously adapted to the HC diet.

Rats tested naively on alternative diets. When rats adapted to the HF diet were tested naïvely on the HC diet (Fig. 2 and Table 2), intracerebroventricular Ent reduced the intake of the test HC diet by 20% at 1 h saline (5.6 ± 0.25 g vs. Ent 4.5 ± 0.14 g, P < 0.05). This effect was still evident at 2 and 4 h on day 1.

In contrast, when rats adapted to the HC diet were tested naïvely on the HF diet, intracerebroventricular Ent did not inhibit intake of the HF diet on this initial (day 1) exposure (Fig. 3 and Table 3).

Retest of Ent responses during adaptation to new diets. Rats that were naïvely tested on the alternate diet (day 1) were maintained on these test diets, and the response to Ent was retested on days 3, 7, 14, and 21. Rats initially adapted to the HF diet and then switched to the HC diet did not respond to Ent when tested on day 3 after introduction of the HC diet (Fig. 2 and Table 2). Rats initially adapted to the HC diet and then switched to the HF diet did not respond to Ent on days 3, 7, or 14 after introduction of the HF diet (Fig. 3 and Table 3). However, on day 21, Ent significantly suppressed intake of HF diet by 28–30% (Fig. 3 and Table 3). The inhibition was evident at 1 h of feeding and lasted for 4 h.

DISCUSSION

The findings presented here show for the first time that the feeding response to Ent is dependent on the composition of the habitual diet rather than the novel diet used at testing time. Observations in the present study indicating that intracerebroventricular Ent reduced intake of HF diet in rats adapted to an HF diet and did not decrease HC feeding in rats adapted to an HC diet are consistent with earlier reports (13, 20, 30). However, two observations suggest that a signal related to the chronic ingestion of dietary fat is required for Ent action: 1) Ent reduced the intake of an HC diet when it was given naïvely to rats previously adapted to an HF diet, but this response disappeared by day 3, and 2) Ent failed to suppress the intake of HF diet in rats previously adapted to the HC diet until 21 days of exposure to the HF diet. These data imply that the signal related to HF feeding disappears quickly after reduction in dietary fat intake but requires considerable time to develop on introduction of high levels of dietary fat.

The present observations that the anorectic response to Ent was present on naive exposure to an HC diet and absent on naive exposure to an HF diet also rule out the possibility that a gustatory or olfactory signal related to dietary fat is essential for the Ent effect on food intake when no choice of diet is available. Thus it is unlikely that Ent affects the immediate feedback system that recognizes the caloric density and palatability of the diet, although Ent does initiate an early satiety response (19). The Ent stimulation of sympathetic nerve firing rate is also only observed in rats that have been adapted to HF diets (26). Because these experiments were performed in anesthetized rats, the results indicate again that presence of fat in the oronasal/pharyngeal areas or the gastrointestinal tract is not necessary for a response to Ent when rats are fed an HF diet.

Table 3. Intake of HF diet by rats previously adapted to HC diet

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Day 1 Saline</th>
<th>Day 1 Ent</th>
<th>Day 3 Saline</th>
<th>Day 3 Ent</th>
<th>Day 7 Saline</th>
<th>Day 7 Ent</th>
<th>Day 14 Saline</th>
<th>Day 14 Ent</th>
<th>Day 21 Saline</th>
<th>Day 21 Ent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>3.8 ± 0.5</td>
<td>3.7 ± 0.2</td>
<td>4.7 ± 0.3</td>
<td>4.5 ± 0.6</td>
<td>5.5 ± 0.3</td>
<td>4.9 ± 0.6</td>
<td>5.4 ± 0.6</td>
<td>5.0 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>1</td>
<td>4.8 ± 0.7</td>
<td>5.1 ± 0.4</td>
<td>6.2 ± 0.9</td>
<td>5.9 ± 0.7</td>
<td>8.2 ± 0.7</td>
<td>7.6 ± 1.1</td>
<td>7.5 ± 0.9</td>
<td>7.5 ± 0.6</td>
<td>7.5 ± 0.3</td>
<td>5.6 ± 0.51</td>
</tr>
<tr>
<td>2</td>
<td>5.8 ± 0.8</td>
<td>5.8 ± 0.5</td>
<td>7.9 ± 0.9</td>
<td>7.4 ± 1.1</td>
<td>9.6 ± 0.9</td>
<td>8.8 ± 1.2</td>
<td>8.9 ± 0.8</td>
<td>7.6 ± 0.6</td>
<td>8.1 ± 0.3</td>
<td>5.8 ± 0.61</td>
</tr>
<tr>
<td>4</td>
<td>5.8 ± 0.8</td>
<td>5.9 ± 0.5</td>
<td>10.2 ± 1.3</td>
<td>8.5 ± 1.1</td>
<td>10.0 ± 1.0</td>
<td>8.9 ± 1.2</td>
<td>9.7 ± 0.8</td>
<td>7.8 ± 0.6</td>
<td>8.2 ± 0.3</td>
<td>6.7 ± 0.3*</td>
</tr>
</tbody>
</table>

Data are expressed as mean cumulative food intake (g) ± SE. ANOVA indicated significant effect of day (F(4,54) = 5.39, P < 0.001), but effects of treatment (F(1,54) = 3.38, P = 0.07) and interactions of day × treatment (F(4,54) = 0.39, P = 0.81) were not significant. *P < 0.05, †P < 0.001 vs. corresponding saline treatment.
diet. However, rats will selectively reduce fat intake in response to Ent when they are provided with a two- or three-choice diet (18–20, 30, 31). This indicates that there must be an association between the taste or smell of fat and the response to Ent that would enable a rat to continue to eat carbohydrates while reducing its intake of fat. The recent identification of a fatty acid-sensitive K⁺ channel on taste buds (16) supports the concept of a fat taste system that might be an important component in a rat's ability to selectively reduce fat intake.

The mechanism through which chronic ingestion of a high-fat diet increases the susceptibility to Ent is not known. Fat feeding increases the synthesis and secretion of the precursor molecule procolipase and Ent from the gastric mucosa (J. Chen and D. A. York, unpublished observations) and exocrine pancreas (28). It is possible that increased secretion of Ent may lead to upregulation of Ent signaling pathways, although its receptors have not yet been identified. This explanation seems unlikely, however, because voluntary intake of fat is inversely related to pancreatic procolipase levels in Sprague-Dawley rats (14), dietary obese Osborne-Mendel rats (30), and genetic obese fa/fa rats (27); i.e., animals with lower endogenous procolipase levels eat more and respond better to exogenous Ent than rats with high levels of procolipase and Ent (27, 30).

Dietary fat ingestion modifies the activity of the endocrine system. Feeding animals on high-fat diets promotes greater weight gain than high-carbohydrate diets (6), and these animals increase leptin (1, 15), insulin (6), and corticosterone secretion (7). Insulin and corticosterone are key endocrine factors in energy metabolism, and both are reported to inhibit procolipase mRNA production (10, 11). Adrenalectomy abolished the feeding response to Ent and increased pancreatic colipase mRNA levels in fa/fa rats (27). Conversely, corticosterone has been shown to promote the feeding response to Ent (25). Thus increased corticosterone secretion might be a prerequisite for Ent activity on feeding behavior.

Leptin secretion from adipose tissue increases when animals are fed an HF diet and also increases as body fat levels rise (1, 15). Leptin is thought to be a feedback signal that maintains energy balance through its central actions to inhibit food intake and promote sympathetically mediated energy expenditure (8). It also suppresses insulin secretion (32). It is possible that leptin, either directly or indirectly, provides the permissive signal to facilitate the response to Ent. This seems unlikely, however, because the obese Zucker fa/fa rat, which has a mutation in the leptin receptor to impair its binding activity (9), is very sensitive to Ent (27). We recognize, however, that leptin does have some effects in fa/fa rats (21) and that a better model to test this hypothesis may be the diabetic db/db mice, which lack the intracellular signaling mechanisms of the leptin receptor (17).

Feeding an HF diet also modifies the activity of sympathetic nerves innervating interscapular brown adipose tissue. The decrease in sympathetic nerve firing rate is observed 22 but not 7 days after introduction of an HF diet (34). This corresponds to the current results that showed that 3-wk adaptation on the HF diet was necessary before the effect of Ent was observed on food intake. The close reciprocal control of food intake and sympathetic activity has been well described, although the relationship of these two responses is unclear (5). However, it is possible that this reduction in sympathetic activity may be an important component that provides the correct internal milieu for Ent action. Metabolic signals associated with HF diets might also provide the permissive signal for Ent action. Perhaps the most obvious possibility is ketone bodies, which increase on feeding of HF diets and are known to act centrally to inhibit food intake (2).

Centrally, Ent appears to inhibit feeding through inhibition of a k-opioidergic pathway (3, 31). Opioids have a wide range of effects on feeding behavior, including a role in the reward/pleasure responses. Because HF diets are palatable and are often preferred by rodents, it is possible that an increase in the activity of a k-opioidergic pathway, associated with chronic ingestion of dietary fat, is necessary for the response to Ent. The association of Ent sensitivity with rat strains that have dietary preferences for fat is consistent with this hypothesis.

Perspectives

The results from this study, along with the previous reports, provide evidence that chronic ingestion of fat is essential for the response to Ent. Ingestion of high levels of dietary fat induces a range of endocrine, metabolic, and neurochemical changes. Because HF diets also increase Ent secretion, it would appear that some induced signal may initiate a feedback system through which Ent might reduce fat intake. Elucidation of such a mechanism would provide further insight into the physiological control of energy balance and might identify some of the underlying differences that determine dietary preference for fat.

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